alanine at [all] the positions designated and all positions therebetween.

In claim 62, line 1, change "61" to --60--.

In claim 63, line 1, change "61" to --60--.

In claim 67, line 1, change "66" to --65--.

In claim 68, line 1, change "66" to --65--.

In claim 73, line 1, change "cindition" to --condition--.

REMARKS

Claims 1, 11, 12, 15-17, 24-28, 30, 31, 58-60, 62-65, 67-73 are pending in this application. Favorable reconsideration of the outstanding objections and rejections as they may apply to the present claims is respectfully requested for the reasons that follow.

Amendments

An obvious typographical error and various informalities have been corrected in the specification, as well as various objectionable terms and a typographical error in the claims.

Claim 1 is amended to recite t-PA variants having an extra Asn-X-Ser or Asn-X-Thr tripeptidyl sequence that starts at an amino acid position selected from a Markush group encompassing the amino acid positions 57 to 61, 63 to 69, 99, 101, 103 to 105, 106, 107, 109, 112 and 250 of native human t-PA, and having N-linked glycosylation attached to the aspartic acid residue within the

tripeptidyl sequence. The t-PA variants encompassed by claim 1 as amended are supported at least on page 10, line 14 to page 11, line 2, page 19, line 10 to page 20, line 8, in Table IV on page 66, and in Example III on page 67 of the specification and in original claim 11. The added N-linked glycosylation of the t-PA variants is supported at least on page 10, lines 14-16, page 11, lines 19-24, page 16, lines 3-24, page 19, lines 23-33, page 20, lines 10-15, and page 48, line 7 to page 51, line 28.

Claim 11 is amended to recite specific t-PA variants supported at least on page 10, line 14 to page 11, line 2, page 19, line 10 to page 20, line 8, in Table IV on page 66, and in Example III on page 67 of the specification and in original claim 11.

Claim 12 is amended to delete recitation of a t-PA variant having an asparagine at amino acid positions 67 and 103 in native t-PA. Claim 12 is also amended to include a t-PA variant having an asparagine at amino acid position 105 and either a serine or threonine at amino acid position 107 of native t-PA. This embodiment of the invention is supported at least on page 67, line 18 of the specification.

Claim 30 is amended to be dependent on claim 1 instead of cancelled claim 29 and to recite t-PA variants with specific alanine substitutions supported at least on page 21, line 25 to page 22, line 7 of the specification.

Claims 62 and 63 are amended to depend from claim 60 instead of cancelled claim 61. Claims 67 and 68 are amended to depend from claim 65 instead of cancelled claim 66. Claim 73 is amended to correct the typographical error "condition" to read "condition."

Drawings

The drawings are objected to in view of various informalities. Appropriate corrections will be made when the Examiner indicates that allowable subject matter exists in this application.

Objection to Informalities in the Specification

The Examiner requests entry of a statement at the beginning of the specification referencing all prior applications and the status of same. As amended herein, the specification now includes the requested statement. The Examiner is correct in assuming that Applicants claim priority under all parent applications pursuant to 35 U.S.C. §120. The Examiner also objects to a typographical error appearing on page 20, line 19 regarding a glycosylation site at amino acid position 210 instead of 218 in human t-PA. Since the specification has now been amended to correct the error, applicants respectfully request reconsideration and withdrawal of the objection to the specification.

Double Patenting Rejection

Claims 1, 11, 12, 19-31, and 58-73 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-7, 9-27, 33, 35 and 37 of copending U.S. Ser. No. 07/894,213. Applicants submit herewith a terminal disclaimer of the portion of any patent that may issue under the present application which extends beyond the life of any patent issued under U.S. Ser. No. 07/894,213. Accordingly, applicants respectfully request reconsideration and withdrawal of the provisional obviousness-type double patenting rejection.

Rejection of Claims 1, 11, 12, 15-31, 58, 59, 61, 63, 66, 68, 70 and 72 under 35 USC §112, first paragraph

Claims 1, 11, 12, 15-31, 58, 59, 61, 63, 66, 68, 70 and 72 are rejected under 35 USC §112, first paragraph because the specification allegedly fails to enable the claimed invention. Since claims 18-23, 29, 61 and 66 are cancelled herein, the rejection is now moot with respect to these claims. Applicants respectfully traverse the rejection as it may apply to the remaining claims.

The Examiner contends that the specification does not support the modification of plasminogen activators other than human t-PA. The Examiner further contends that the specification does not enable O-linked glycosylation variants of plasminogen activators. With respect to claim 1, the Examiner argues that the specification fails to support the broad spectrum of modified plasminogen activators encompassed by the claim. Without intending to acquiesce to the Examiner's position, applicants herein amend claim 1 to recite specific N-linked glycosylation variants of human t-PA.

The Examiner alleges that claim 15 is not enabled because the specification does not describe finger domains in plasminogen activators other than t-PA. The Examiner suggests that applicants intended to direct claim 15 to t-PA. Since claim 1 (from which claim 15 depends) is amended herein to recite human t-PA variants, claim 15 now recites human t-PA variants as suggested by the Examiner.

The Examiner maintains that claim 18 is not enabled on grounds that the specification provides no guidance on the modifications to regions 205-215, 233-242 and 244-255 recited in claim 18. Without

acquiescing to the rejection, but rather to expedite prosecution, applicants have cancelled claim 18.

The Examiner rejects claims 19-23 on grounds that the specification does not provide sufficient support for plasminogen activator mutations conferring resistance to enzymatic cleavage. To advance prosecution, claims 19-23 are cancelled herein.

The Examiner alleges that undue experimentation would be required to make the t-PA protease domain alterations that confer zymogenic activity as claimed in claims 29 and 30. To expedite prosecution, applicants have cancelled claim 29 and amended claim 30 to depend from claim 1. Thus, claim 30 no longer incorporates the "alterations" or "zymogenic activity" language of claim 29.

Finally, the Examiner argues that the modifications to positions 296-299 recited in claims 30, 61 and 66 are enabled only as to the substitution of alanine at each of positions 296-299. Without intending acquiescence to the rejection, applicants have cancelled claims 61 and 66 and amended claim 30 to recite the alanine substitutions specifically exemplified in U.S. Patent No. 5,108,901 which the present specification incorporates by reference.

In view of the present amendments and the discussion above, applicants submit that the present claims are fully supported by the specification as filed and respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Rejection of Claims 1, 11, 58 and 59 under 35 USC §103

Claims 1, 11, 58 and 59 are rejected under 35 U.S.C. §103 as allegedly being obvious over U.S. Patent No. 4,766,075 by Goeddel *et al.*, hereafter "Goeddel." The Examiner argues that Goeddel suggests amino acid modifications or substitutions to human t-PA which do not alter the biological activity of human t-PA. The Examiner adds that the claims pending in the present application cover t-PA variants which have not been shown to possess altered biological activity and concludes that such embodiments of the invention are obvious over Goeddel. She specifically notes that there is no evidence of record that the variants N64S66 t-PA, N64T66 t-PA, and N250 t-PA have an altered biological activity. Finally, according to the Examiner, not every tripeptidyl sequence (glycosylation signal) in the t-PA variants of the present invention actually results in glycosylation.

Applicants respectfully traverse the rejection as it may apply to the present claims.

Goeddel discloses the cloning and expression of DNA encoding native human t-PA. The t-PA variants claimed in claim 1 of the present application are characterized by having extra N-linked glycosylation sites not present in native t-PA and by having extended in vivo half-lives (slower plasma clearance rates) as compared to native human t-PA. There is nothing in Goeddel that would suggest this unexpected finding.

To complete the record, enclosed is a copy of the Declaration of Dr. Bruce A. Keyt under 37 C.F.R. §1.132 ("the Keyt Declaration"), which was filed on November 23, 1993 in connection with copending application U.S. Serial No. 08/036,014. Dr. Keyt is a named inventor in the present application and is an expert in the

field of thrombolytic protein biochemistry, having worked in this area for over 11 years.

In paragraph 3 of the Declaration, Dr. Keyt presents data showing that N250 t-PA is cleared from the bloodstream at a rate that is only 42% of the native human t-PA clearance rate. In paragraphs 4 and 5, Dr. Keyt demonstrates that the addition of N-linked glycosylation anywhere in the areas defined by amino acid positions 57 to 61 or 63 to 69 of native t-PA is expected to result in a t-PA variant with a significantly lower plasma clearance rate than that of native t-PA.

Table 1 and Fig. 1, attached as Exhibits B and C, respectively, of the Keyt Declaration, provide evidence of the significantly lower plasma clearance rates of t-PA variants with an extra N-glycosylation site starting at amino acid position 60, 67, 99, 101, 103, 104, 105, 106, 107, 109, 112, or 250 of native t-PA. Dr. Keyt states that from these data it is apparent that positions 101 to 107 of the t-PA molecule form a region in which the addition of an N-glycosylation site produces a plasma clearance rate that is at least 50% lower than the clearance rate of native human t-PA.

Fig. 1 also shows that movement of the N-glycosylation site two or five amino acid positions downstream from the C-terminal end of the 101-107 amino acids region or two amino acid positions upstream from the N-terminal end of the region results in t-PA variants with clearance rates that are still significantly lower than that of native human t-PA. See paragraph 4 of the Keyt Declaration.

Given the behavior of N-glycosylation sites in the 101-107 region, Dr. Keyt expects that N-linked glycosylation added anywhere within the region defined by amino acid positions 60 to 67 will

result in a significantly lower plasma clearance rate than that of native human t-PA. Furthermore, Dr. Keyt provides reasons why a practitioner would reasonably conclude that a t-PA variant N-glycosylated at a site anywhere between amino acid positions 57 to 61 or 63 to 69 as claimed herein would have a significantly lower plasma clearance rate than native human t-PA. See paragraph 5 of the Keyt Declaration.

In view of the experimental evidence of record, the invention claimed in claim 1 of the present application is submitted to be unobvious over Goeddel. The Examiner's concern that the t-PA variants claimed herein might not, under all circumstances, be actually glycosylated at the indicated positions is believed to be irrelevant to the patentability of the claimed invention. As amended herein, the present claims are drawn to t-PA variants possessing N-linked glycosylation at the indicated positions. Moreover, the overall teaching of the present application is that glycosylation at certain sites of native human t-PA that are not ordinarily glycosylated results in variants that have an extended circulatory half-life and slower clearance rate compared to native human t-PA (see, e.g., page 11, lines 19-24 of the specification.) It is well within the skill of an ordinary artisan to ensure that glycosylation is indeed present at those sites, as taught by the present invention.

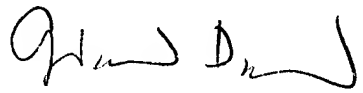
Given that the t-PA variants claimed in claim 1 are patentable over Goeddel as shown above, it follows that dependent claim 11, drawn to particular t-PA variants, and dependent claims 58 and 59, drawn to compositions comprising the t-PA variants of claim 1 and to methods of using such compositions, respectively, are also patentable over Goeddel.

In light of the foregoing arguments and the Keyt Declaration, reconsideration and withdrawal of the rejection of the claims under 35 USC §103 is respectfully requested.

Applicants believe that this application is now in condition for allowance and respectfully request a Notice to that effect.

Respectfully submitted,

GENENTECH, INC.



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